Run-off Patterns of Vancomycin Resistant Enterococci (VRE clones) in Cows Raw Milk and Imported Milk Powders at Baghdad Markets

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ABSTRACT

Codebook shifting of some clones of *Enterococci* from normal flora to genotypically and phenotypically foodborne opportunistic and multidrug resistant pathogens in our ecosystem represent a dangerous hygienic problem. Seventy-five pooled milk samples were collected from different districts in Baghdad ecosystem from January until May (2019). Fifty raw milk samples pooled directly from cows and milk containers (25 each) and 25 imported milk powders pooled from Baghdad markets, in which 15 samples were inspected monthly (five from each brand). Certified and modified traits profits for isolation and confirmation policy. Frequency and distribution of verified data in Baghdad environment revealed variable results. Seven *Enterococcus faecalis* phenotypes (9.33%) were recovered from raw milk samples, in which four isolates (5.33 %) recovered from cows raw milk and three isolates (4 %) recovered from milk containers. No clones were found in imported milk powders. All isolates were biofilm-producers and vancomycin resistant as verified via modified Christensen biofilm assay and antibiotics sensitivity test. Monitoring these contamination events with vancomycin resistant *Enterococcus faecalis* needs sophisticated hygienic efforts in Baghdad ecosystem.

Keywords: *Enterococcus faecalis*, Vancomycin, Resistant, Cow, Milk

Introduction

Foodborne multidrug resistant pathogens are a major cause of foodborne diseases and food poisoning and thus pose a serious threat to food safety. In recent years, diseases caused by foodborne pathogens have become an important public health problem in many parts of the world, producing a significant rate of morbidity and mortality.

According to a World Health Organization report, foodborne diseases are considered as an emergent public health problem in both developed and developingcountries. In addition, many other outbreaks of pathogens have been found to be outbreaks of pathogens have been found to be associated with biofilms. Bacteria can easily adhere to the surface of food materials, food processing equipment, and the surface of pipelines, and can eventually form a bacterial biofilm (1, 2).

Enterococci are ubiquitous microbiota found in the normal intestinal flora of humans and animals, and are common in environments contaminated by human and animal fecal materials.

They have been recovered readily from foods, such as milk and meat products and various environmental sources. Inadequate milk hygiene ecosystem encourages proliferation of these mediators. *Enterococci* enter and colonize the mammary gland through the ducts and generate infection.

Vancomycin resistant *Enterococci* (*VRE* clones) are currently emerging as a global threat to public health (3). Glycopeptide antibiotics are currently the only cure for *Enterococcus*-originated infections, which are resistant to ampicillin and aminoglycoside antibiotics.

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Meanwhile vancomycin and teicoplanin resistant *Enterococci* are discovered, its population has continuously increased, therefore the use of glycopeptide-related antibiotics should be strictly prohibited, and otherwise infection resulting from multiple drug-resistant *Enterococcus* will be impossible to treat (4).

VRE clones are considered as emerging pathogens of humans and are often associated with hospital-acquired infections, such as endocarditis, urinary tract infections, bacteremia and neonatal sepsis, in which *E. faecalis* bear the principal responsibility. The prevalence of *Enterococci* with acquired antibiotic resistance is high in animals and humans regularly treated with antibiotics (5).

Enterococci, along with *Escherichia coli*, are used as indicators of fecal contamination in water courses, as well as in food, and can serve as indicators of hygiene process and food and drinking water quality.

Although *Enterococci* are recognized as conferring beneficial properties to fermented milk products, if they are present in dairy foods after manufacture, they may pose food safety risks as potential pathogens and reservoirs of antibiotic resistance and contribute to food spoilage.

Consequently, the importance of identifying sources of *Enterococcal* contamination in milk supplies and risk factors for persistence in manufactured products has received an increasing interest (6).

Enterococci are not only intrinsically resistant to several antibiotics, but are also characterized by a potent and unique ability to exchange genetic material.

In addition, selective pressure exerted by the use of antibiotics as growth promoters in food animals appears to have created large reservoirs of transferable antibiotic resistance in various ecosystems. With the emergence of glycopeptide resistance in *Enterococcus faecium* outside hospitals, a large reservoir of transferable resistance (*vanA* gene cluster) was identified in animal husbandry due to the use of avoparcin as a feed additive.

The spread of resistance, which enters the human *Enterococcal* flora via the food chain and the transfer of this trait to pathogenic species (i.e. the recent emergence of *Staphylococcus aureus* with decreased sensitivity to vancomycin) indicate the need for greater control of the use of glycopeptide antibiotics in animal feed.

Therefore, the barrier separating *Enterococci* as dangerous contaminants from pathogens appears to be most fragile (7). The objective was to investigate the occurrence of Vancomycin Resistant *Enterococcus faecalis* (*VRE* clones) in cows raw milk and imported milk powders at Baghdad markets.

Materials and Methods

Modified practical experience designed guidelines were authorized (8-11).

Seventy-five pooled samples were collected from different districts in Baghdad ecosystem from January until May (2019). Fifty raw milk samples were assembled directly from cows and milk containers (25 each), and 25 imported milk powders were assembled from Baghdad markets, in which 15 samples were inspected monthly (five from each brand). Sample volume was 50 ml pooled directly from cows and collected from milk containers, and 50 g were collected from each imported milk powders.

Certified and modified traits profit for isolation and confirmation policy.

All samples were inoculated and diluted as 25 ml or g from each brand in 125 ml of tryptone soya yeast extract broth. They were mixed well manually, incubated at 37 $^{\circ}$C for 24 hours, and then cultured on sodium azide tryptone soya yeast extract blood agar for 2-3 days.

The *Enterococci* mean log count was enumerated via the formula: mean number of colonies on cultured plate x a reciprocal dilution factor x 50 cfu/ml. Segregation confirmation strategies were followed (12-14).

Gram stain, India ink capsule stain, catalase test, buffered sodium pyruvate, bile aesculin, and MacConkey growth with Lancefield grouping by latex agglutination system aided in confirmation strategy. Modified Christensen Biofilm assay was used for detection of biofilm, in which tryptone soya yeast extract broth and wide holes tissue culture plates were used for detection of slim-producers. Kirby-Bauer disc diffusion method via Muller-Hinton agar and vancomycin discs (VA 30 µg) was used for determining the sensitivity profile of isolates. Data were subjected to Chi-square analysis using the Statistical Package for the Social Sciences (SPSS, version 25) (15), and $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Seven *Enterococcus faecalis* phenotypes (9.33%) were recovered from raw milk samples, in which four isolates (5.33 %) were recovered from cows raw milk and three isolates (4 %) recovered from milk containers. No clones were found in imported milk powders.

Recovery according to brands and season is represented in Tables 1 and 2, while *Enterococci* counting is represented in Table 3. Growth and hemolysis patterns of *E. faecalis* on modified sodium azide blood agar are represented in Figure 1.

Table 1. Recovery of *E. faecalis* from total brands samples

Sample Brand	Number	Positive Recovery %
Cows Raw Milk	**25**	**4 (5.33 %) [A]**
Milk Containers	**25**	**3 (4 %) [A]**
Milk Powders	**25**	**None [B]**
Total	**75**	**7 (9.33 %)**

[A,B] Indicate significant differences in recovery percentages vertically among brands at level (P≤0.05)

Table 2. Recovery of *E. faecalis* according to season

Month	Positive Recovery %
January	**2 (2.66 %) [A]**
February	**2 (2.66 %) [A]**
March	**1 (1.33 %) [B]**
April	**1 (1.33 %) [B]**
May	**1 (1.33 %) [B]**

[A,B] Indicate significant differences in recovery percentages vertically among recovery months at level (P≤0.05)

Table 3. Mean log count of *E. faecalis* (cfu/ ml) from total brands samples

Sample Brand	*E. faecalis* (cfu / ml)
Cows Raw Milk	**2.397[B]**
Milk Containers	**3.698[A]**
Milk Powders	**None[C]**

[A,B,C] Indicate significant differences in mean log count vertically among brands at level (P≤0.05)

Figure 1. Growth and hemolysis pattern of *E. faecalis* on modified sodium azide blood agar

Multidrug resistance foodborne pathogens have been encountered in many studies (1, 16).

Significant and important differences appeared in recovery percentages and log count of *E. faecalis* from each brand, in which pooled raw milk samples from cows showed the highest recovery percentages with the lowest count versus raw milk samples collected from milk containers which showed the lowest recovery percentages and the highest count.

Clean imported milk powders indicate no contamination features. Both raw milk brands showed unacceptable levels of contamination with these dangerous isolates that alert public health policy.

Most isolates were recovered during cold season, and this might be attributed to stress conditions, environmental temperature, and ecosystem of

collected brands. These findings are consistent with those illustrated in many literatures (16-24).

All isolates were biofilm-producers, and vancomycin resistance was verified via modified Christensen biofilm assay and Kirby-Bauer disc diffusion method antibiotics sensitivity test. Dual shelled barriers biofilm production with capsule protected these clones from antibiotics and environmental stressors, and in the same time induced hidden stress hardening and quorum sensing bionetworks for controlling their ability to live in versatile conditions.

Hidden subclinical cases of mastitis with infectious recalcitrant foci and accumulative slim matrix in milk containers aid in persistent and recycled contamination in our ecosystem with vancomycin resistant *E. faecalis* clones that affect economic and public health. Monitoring these contamination events with vancomycin resistant *E. faecalis* needs sophisticated hygienic efforts in Baghdad eco-system (16-24).

Conflict of Interest

The authors declare that there is no conflict of interest.

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أنماط جريان المكورات المعوية المقاومة للفانكومايسين في الحليب الخام للأبقارومساحيق الحليب المستوردة من أسواق بغداد

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الخلاصة

يمثل تغيير الشفرات لبعض العتر المستنسخة من المكورات المعوية من الحالة الطبيعية إلى مسببات الأمراض الانتهازية وراثيا المنتقلة عن طريق الأغذية في النظام البيئي مشكلة صحية خطيرة. جمعت خمسة وسبعون عينة حليب مجمعة من مناطق مختلفة في النظام البيئي ببغداد من يناير حتى مايو (2019). تم تجميع خمسين عينة من الحليب الخام مباشرة من الأبقار وحاويات الحليب (خمسة وعشرون لكل منهما) وخمس وعشرين من مساحيق الحليب المستوردة المجمعة من أسواق بغداد حيث تم فحص خمسة عشر عينة شهريًا (خمس من كل علامة تجارية). اعتمدت طرائق مقايسة مختبرية محورة للصفات المعتمدة والمعدلة لسياسة العزل والتأكيد. كشف تواتر وتوزيع البيانات التي تم التحقق منها في بيئة بغداد عن نتائج متغيرة. شخصت سبعة أنماط ظاهرية للعزلة البرازية المعوية (9.33٪) من عينات الحليب الخام، حيث استردت أربع عزلات (5.33٪) من حليب الأبقار الخام وثلاث عزلات (4٪) تم استعادتها من حاويات الحليب. لم يتم العثور على العتر المستنسخة المستردة في مساحيق الحليب المستوردة. كانت جميع العزلات منتجة للأغشية الحيوية وتم التحقق من مقاومتها للمضاد الحيوي الفانكومايسين من خلال اختبار الاغشية الحيوية القياسي المعدل واختبار حساسية المضادات الحيوية. رصد ومراقبة حالات تلوث الأغذية بهذه العتر المقاومة للفانكومايسين يحتاج إلى جهود صحية متطورة ومحورة في النظام البيئي ببغداد.

الكلمات المفتاحية: المسبحيات المعوية البرازية، فانكومايسين، المقاومة، الحليب، الأبقار